Growth of the Mouse Corpus Callosum

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Wahlsten, D. Growth of the mouse corpus callosum. Developmental Brain Research, 1984, 15, 59-67.

Abstract:

Brains of BALB/cCF inbred mice were examined at 15 ages ranging from 16.5 to 50.5 days from conception and cross-sectional areas of major forebrain fibre tracts at the midsagittal plane were measured. The anterior commissure appeared prior to the corpus callosum (CC), which was first seen at midplane at 17.0 days, and both tracts underwent a very rapid increase in size in the prenatal and early postnatal period, reaching the adult range of size at about 1 week after birth or several days prior to the onset of myelination. The growth spurt of these fibre tracts was much more pronounced than that of whole brain. By comparing BALB/c mice with hybrid mice that always have normal CC, it was found that some BALB/c mice at 18.5 days of age which have very small or absent CC do so because the growth of the whole brain is retarded whereas others have CC that is small for the brain size. Evidence also suggested that many mice with no CC but normal brain size at 18.5 days prenatally do eventually acquire at least a small CC. Observations at 3 postnatal ages of BALB/c mice weighed and marked at birth revealed that the 'runts' with low birth weight, which were presumably retarded prenatally, either die or catch up with mice of normal birth weight and do not have unusually small adult CC.

Key words: anterior commissure —brain weight — prenatal development — BALB/c — fetus — corpus callosum

Article:

INTRODUCTION

Hereditary absence or gross deficiency of the corpus callosum (CC) has been documented for two inbred strains of mice[22], and inheritance of the defect has been shown to depend on more than a single Mendelian gene in BALB/c mice[23]. Because the anatomical and functional consequences of failure of so many axons to cross between the hemispheres undoubtedly depend to some extent on the time in development when the failure occurs, a study of the normal time course of growth of the CC was done in order to identify the periods in brain development when processes leading to the defect are most likely to be operating.

Provided there is no major axonal degeneration involved in the hereditary defect, cases of total absence of CC clearly must occur because of interference with things which normally guide or propel the growth of axons between the cerebral hemispheres, and this interference must occur prior to the time the axons reach the region of fusion of the hemispheres. The time when CC axons first cross the mid-sagittal plane has been documented in 3 previous studies of mice. in two kinds of hybrid mice CC is present at about 16 or $16\frac{1}{4}$ days after conception[3,21]. In inbred C57BL/6J mice it has been first noted at about 161/2 days[21] or 17 dayst[3] after conception by different investigators using somewhat different breeding and histological methods, The CC of BALB/c mice first appears 12-24 h later than that of C57BL/6J mice[21], part but not all of this strain difference in timing of fibre tract growth resulting from a difference in rate of growth of the whole animal which is evident from its external morphology and body size[24], Thus, CC first appears at least two days prior to birth in most laboratory mice, and total absence of CC is certainly a defect of prenatal origin.

Among adult mice with a defective CC, those with total absence of transcortical fibres are a small minority[22]. Much more common is a CC that is well below normal in cross-sectional area but nonetheless contains

thousands of axons connecting the two hemispheres. For some reason the CC begins to form in these animals but stops accumulating axons before the normal adult CC size is attained. It is very likely that the local events causing partial deficiency of CC rye somewhat different from those causing total absence of CC. with respect to both timing and site of action of these processes in the brain.

Very little is currently known about the early growth of the mouse corpus callosum after the pioneering fibres traverse midplane. Silver et al.[13] noted that the lengths of CC at midplane in C57BL/6J mice are about 300 and 450 μm at 17 and 18 days after conception, respectively, whereas it averages 1.2 min on the day of birth and 2.0 mm 1 week after birth. The first myelin sheaths in CC at midplane appear about 11 days after birth", and staining of myelin at the same location with a lipid stain is clearly evident by about 14-16 days after birth[19], depending on the strain of mouse[26]. It is not clear whether the major growth phase of the mouse CC is mainly completed before myelination begins or whether significant numbers of axons continue to cross midplane throughout the period of myelination.

Research with laboratory rats is suggestive of a phase of rapid postnatal growth in CC in which many axons have already crossed midplane at birth, entered the cerebral cortex within 5 days of birth and established an adult-like pattern of connections by 9 days in somatic sensory cortex[26] or a complete adult pattern by 15 days after birth in parietal cortex[6]. However, these recent studies of topographical organization have not quantified the overall size of CC at various ages. An earlier study of postnatal CC growth[15] indicated a gradual increase in cross-sectional area of rat CC from birth to about 100 days of age, a result which is difficult to understand in the context of recent studies suggesting very rapid growth of CC in the neonatal period.

The present study therefore began with measurements of fibre tract sizes in BALB/cCF mice at a wide range of ages extending from just before the appearance of CC prenatally to the time of weaning postnatally when CC is close to its adult size. Results of this study indicated that prior to birth many mice appear to have very small CC because the growth of the whole mouse is retarded. Consequently, in the sec-mid phase of the study, several litters of hybrid mice which never have deficiency of CC were measured in order to provide art independent standard of normal brain growth in the late prenatal period and objective criteria of developmental delay and CC deficiency.

This investigation confirmed the existence of retarded development in certain BALB fetuses and led to the question of whether the retarded animals might be the ones which later exhibit partial absence of CC. This possibility was assessed in the third phase of the study wherein individual animals were weighed and marked at birth and then tested later to see if the mice retarded or 'small for date' at birth would catch up with their littermates in behavioral and brain development.

MATERIALS AND METHODS

All mice examined in the study were conceived and reared in the author's laboratory under environmental conditions described previously[22]. The BALB/cCF strain had been maintained by full-silt matings for at least 4 generations in the mouse colony at Waterloo and had been inbred for at least 100 generations prior to their acquisition from Carworth Farms in 1976 and 1977. The B6D2F_1/J hybrid mice (C57BL/6J mother and DBA/2J father) were procured from the Jackson Laboratory, Bar Harbor, ME. All females were virgins and at least 10 weeks old when they were mated to produce offspring for the present study. The BALB/cCF females were a always mated with a brother, but the B6D2F_1/J females were mated to a male who might not have been a littermate.

Phase I

In the first phase 44 litters containing $2^4$5 live mice were obtained front timed pregnancies of BALB/cCF mice. Male and female siblings were separated at weaning (1 month after birth) and housed in same-sex groups until mating, whereupon one male was housed continuously with 1, 2 or 3 of his sisters and the females were checked twice daily for the presence of a vaginal plug. The beginning of life (0.0 day) was defined as the midpoint between the detection of a plug and the previous check when there was no plug. After pregnancy was

confirmed by increased body weight of the mother, the entire litter was assigned to an age for testing in a manner that was convenient for the experimenters (i.e. avoiding weekends and certain evenings, when possible). Matings were continued until at least two litters containing a total of at least 10 mice were sacrificed at each of the gestation ages listed in Table I. At 18.5 days extra litters were tested in order to be sure that a few mice with deficient CC would be detected prenatally. For gestation ages of 19.0 days or less the pregnant female was anesthetized with pentobarbital sodium and perfused intracardially with neutral buffered 10% formalin, Each fetus was rapidly removed from the uterus and surrounding membranes, immersed in fixative, blotted to remove excess fluid and weighed to the nearest mg. The scalp and skull were then cut away and the exposed brain was immersed in fixative for 24 h, at which time it was removed completely from the head, left in fixative for at least 1 week, and later blotted and weighed to the nearest mg. For gestation ages 19.5 days or older the mother was allowed to give birth and suckle her pups until the time of sacrifice, when each pup was weighed, anesthetized with chloroform vapour and perfused intracardially with fixative. The brain of each mouse was then extracted from the skull and fixed for at least 1 week prior to blotting and weighing. After fixation in formalin for at least 2 weeks, each brain was encased in a mixture of 15% gelatin and 15% glycerin in distilled water, and the gelatin block was allowed to harden in the fixative for at least 48 h. Frozen sections were then cut at 25 µm in the sagittal plane and stained with hematoxylin and eosin. Gelatin embedding and frozen sections were used in order to minimize the shrinkage of brain tissue during histology and yield an accurate estimate of sizes of fibre tracts. The mid-sagittal section was identified separately for CC and CA because the actual plane of cutting was not always exactly sagittal, The outline of each structure was traced and then cross-sectional area in square min was determined as described previously[22].

TABLE I

Number of litters (no. L), total animals (n) and measures of brain and body at 15 gestation ages

Gestation age	No. L	n	% with no CC	Median CC area (mm²)	Median CC length (mm)	CA area (mm²)		Brain weight (g)		Body weight (g)	
						Mean	S.D.	Mean	S.D.	Mean	S.D.
16.5	2	13	100	–	–	0.009	0.003	0.050	0.005	0.49	0.08
17.0	3	13	54	0.010	0.20	0.017	0.005	0.070	0.005	0.60	0.08
17.5	3	26	19	0.086	0.51	0.028	0.004	0.069	0.006	0.77	0.09
18.0	6	21	19	0.121	0.56	0.035	0.010	0.079	0.010	0.84	0.23
18.5	6	35	14	0.236	0.89	0.045	0.008	0.081	0.007	1.02	0.14
19.0	3	16	0	0.244	0.97	0.048	0.007	0.088	0.009	1.06	0.18
19.5	2	14	0	0.424	1.30	0.059	0.009	0.101	0.007	1.42	0.12
20.5	2	10	0	0.420	1.28	0.067	0.008	0.108	0.014	1.46	0.32
21.5	3	16	0	0.620	1.56	0.094	0.021	0.151	0.011	2.17	0.22
23.5	3	13	0	0.851	2.00	0.128	0.014	0.197	0.010	2.75	0.26
25.5	2	12	0	0.928	2.45	0.134	0.027	0.241	0.022	3.50	0.96
30.5	3	14	0	0.994	3.11	0.156	0.018	0.388	0.020	6.66	1.31
35.5	2	13	8	0.889	3.27	0.161	0.008	0.455	0.007	7.92	0.65
40.5	2	13	0	0.974	3.13	0.175	0.013	0.486	0.006	8.78	1.30
50.5	2	16	0	1.056	3.38	0.172	0.020	0.505	0.015	16.19	1.94

Phase 2

136D2F$_1$/J hybrid mice were mated and their F$_2$ hybrid offspring were measured using the same methods presented above. In order to obtain mice having approximately the same average brain and CC sizes as well as range of CC sizes as the BALB/cCF mice at 18.5 days chronological age, hybrid litters were tested at ages 17.0, 17.5 and 18.0 days after conception. This was necessary because these F$_2$ hybrid mice generally develop faster and with less variability than inbred BALB/c mice[24], For analysis of data in this phase of the study, the sample of BALB/cCF mice at 18.5 days was expanded by pooling the data from Phase 1 with similar data on 8 litters collected in the same laboratory by Wainwright and Stefanescu[25] using nearly the same methods. The 8 litters were in a control group which was fed a 27% protein diet having casein as the source of protein; otherwise methods were identical. The body and brain sizes of the 54 mice in these 8 litters sacrificed at 18.5 days did not differ significantly from their counterparts studied in Phase 1 of the present study.

Phase 3

An additional 19 litters of BALB/cCF mice containing at least 6 animals each were obtained from untimed pregnancies using coatings done so that most births occurred in the morning on weekdays. When a mother had

completed giving birth to all her pups and had cleaned and assembled them into the nest, each pup was weighed to the nearest mg and marked on one, two or three limbs with blue ink from a Cadomarkt felt pen. It was necessary to reinforce these marks daily for the first 4 or 5 days after birth because the mother often cleaned her pups so well. When the white hair was long enough, it was dyed with a pattern of one, two or three small black dots on the limbs using Lady Clairol Ultra Blue hair dye which needed reinforcement only once every month or two, The mother mice readily accepted and nursed pups marked with these methods. At 12 days after birth 6 litters were tested for degree of reflex development using a battery of 14 tests described in detail previously[19] and then were perfused as in Phase 1 within 1 h of reflex testing. Another litter containing 10 mice that were obviously retarded in growth was tested at 13 days instead of 12 in order to provide approximately the same average scores as the 12-day animals, and its data are included in the I2-day group for purposes of analysis. Another 6 litters were perfused at 22 days after birth, and 6 more were perfused at either 100 or 101 days after birth. All brains were cut sagittally at 25 μm on a freezing microtome. For the 12-day brains alternate sections were stained with cresyl violet and Sudan Black B, whereas the 22- and 100-day brain sections were stained with metachromatie thionin. The extent of myelination in the 12-day brains was scored from the Sudan Black **B** intensity for 28 fibre tracts using methods described previously[19].

RESULTS

Phase I

Summary statistics for mice at 6 prenatal and 9 postnatal ages are presented in Table I. Median values of cross-sectional area of CC are provided for mice with some CC present instead of means because CC size was sometimes abnormally small in a few mice but no unequivocal criteria for abnormality were available at each age. The median is less influenced by a few deviant scores than is the mean, It is evident that measures of brain growth showed very little difference between certain ages such as 19.5 and 20.5 days, whereas at other ages there was a large advance. Given the small number of litters at most ages and the presence of significant differences between mean scores of litters at the same age in some instances, it would be hazardous to interpret these departures from a smooth growth curve as evidence for developmental spurts and lags. The data provide a good picture of the general progress of CC growth in relation to growth of CA, whole brain and body, especially when these measures are compared in terms of the percent of the 50-day value achieved at each age (Fig. 1).

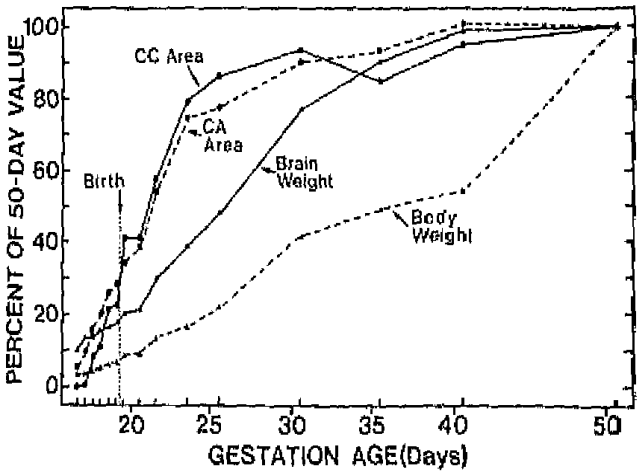

Fig. 1. Average percentage of the 50-day value of 4 variables achieved at each of 15 ages timed from conception. Birth occurred between 19.0 and 19.5 days, and 50 days gestation age was about 30.5 days after birth.

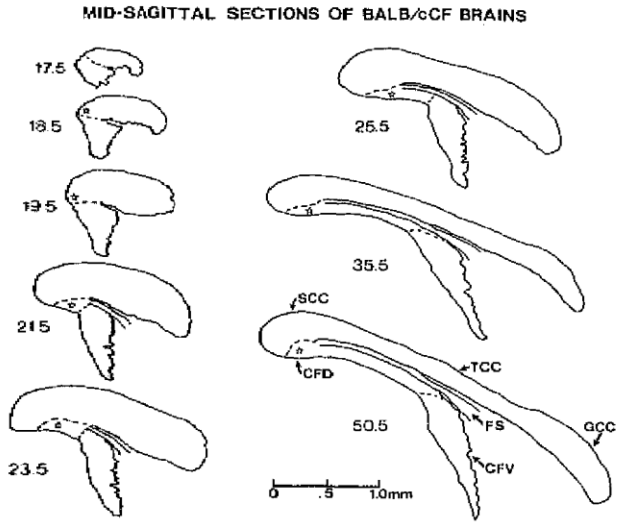

Fig. 2. Outlines of major forebrain fibre tracts drawn from mid-sagittal sections of brains with tract sizes near the median of all normal animals at each of 8 gestation ages ranging from 17.5 to 50.5 days. Dotted lines show the approximate borders between the commissura fornicis ventralis (CFV), commissura fornicis dorsalis (CFD) and corpus callosum (CC). Open stars indicate the approximate locations of CFD. A precise line between CC and CFD could not be determined at 18.5 and 19.5 days using the present methods, although CFD was clearly present at mid-plane. Other abbreviations: FS, fornix superior; GCC, genu corporis callosi; SCC, splenium corporis callosi; TCC, truncus corporis callosi.

There has been much discussion of the postnatal growth spurt in whole brain size[2,4], but whole brain growth clearly proceeds at a slower pace compared to the spectacular progress of growth of CC and CA which reach the adult range of size by 25.5 days gestation age, a mere 6 clays after birth and about a week before the beginning of myelination of the mid-sagittal portions of CC. In a previous study[22] it was concluded that adult mice at 250 days after birth have a normal CC if its cross-sectional area exceeds 0,85 mm^2. In the present study using the same strain of mice, most animals had CC size greater than this criterion only 6 days after birth, a time when brain weight was about half its adult value, body weight averaged 3.5 g, eyes and ears were unopened, and behavior was limited to a few simple reflexes.

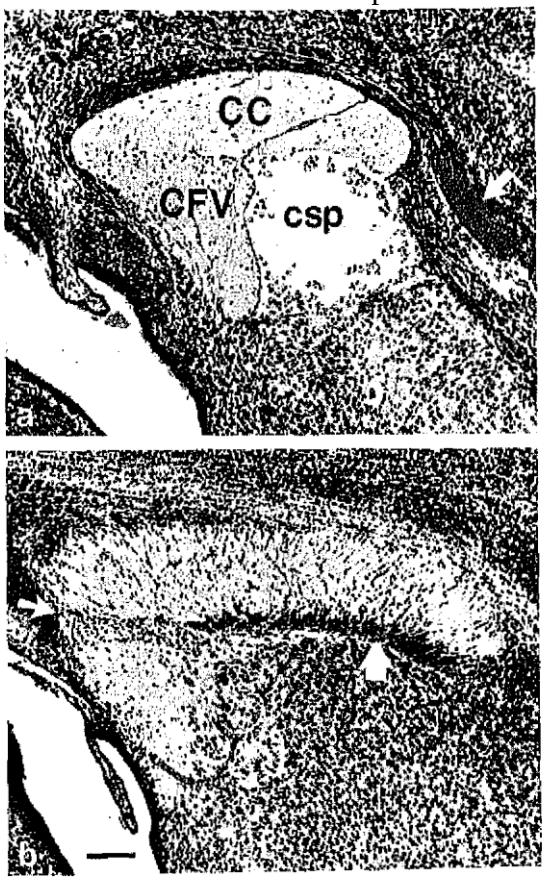

Fig. 3. Sagittal sections of a normal BALB/cCF brain at 18.0 days gestation age embedded in paraffin, sliced at 10 μm thickness and stained with hematoxylin and eosin. a: a midsagittal section showing locations of CC and CFV as well as the middle cerebral artery (arrow). The gap ventral to CC is the cavum septi pellucidi (csp). Bar indicates 0.1 mm. Anterior is to the right. b: a section approximately 250 μm lateral to midplane showing a clear line of demarcation between CC and CFV (small arrow) and a closely packed band of cells ventral to CC (large arrow) known as the glial sling.

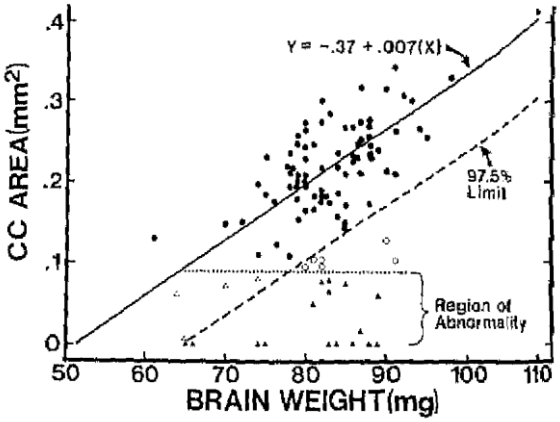

Fig. 4. Line of best fit and 97.5% one-sided confidence limit for relation between CC cross-sectional area (Y) and brain weight (X) of $B6D2F_2$ hybrid mice at 17.0, 17.5 and 18.0 days gestation age. Points represent individual BALB/cCF mice at 18.5 and 19.0 days gestation age. Solid circles are mice considered normal. Open circles are mice considered to have CC size unusually small for their brain weights but not less than 0.08 mm^2, a criterion for abnormality. Solid triangles are mice with CC sizes which are clearly abnormal for their brain sizes. Open triangles are developmentally retarded mice which have abnormally small CC for their chronological age but not with respect to their brain sizes.

During ontogeny the CC changes in shape as well as site as indicated in Fig. 2. Transcortical fibres first appear slightly anterior and dorsal to the hippocampal commissure (CFV) at 17.0 days after conception, and expansion of CC appears to occur mainly in the anterior direction for the next 2 days. At the posterior end of CC there is difficulty delimiting it from CFV. The border indicated in Fig. 2 was found by observing the orientation of groups of glial cells and bundles of axons at midplane and then following the patterns of cells and axons through serial sections until an obvious distinction between CC and CFV was seen (Fig. 3b). This became a more difficult task when the dorsal commissure of the fornix (CFD) was present because CFD and CC do not soon part company as they progress laterally. The CFD was present at midplane in a few fetuses at 18.0 days and all normal fetuses at 18.5 days, but it was very difficult to perceive a sharp border between CFD and CC at midplane. The patterns of glia and axon bundles were sometimes adequate to draw a line when combined with knowledge of the location of the CFD in adults where it stains darkly with a myelin stain. After 20.5 days, growth of CC in the posterior direction was apparent and the structure became thicker throughout its length. The

CC from 21.5 to 25.5 days is remarkable for its thickness in the region dorsal to CFV which is occupied by the truncus of CC and is usually quite thin in normal adults. Although it is not likely that a large proportion of the total axons accumulated by CC arrives at midplane after 25.5 days, the structure does change its overall shape, becoming longer and thinner in most adults.

The ventral portion of the young CC is usually lined with a band of darkly staining cells anterior to CFV that Silver et al.[13] have identified as primitive ghat cells and termed the 'sling'. This band of cells is easily seen in parasagittal sections (Fig, 3b) in all mice from ages 17.0 to 20.5 which have a normal CC. In brains older than 21.5 days it is difficult to perceive the sling as a distinct structure, although some of its cells may still be present but dispersed. Prenatally, the sling is normally continuous with the ventricular layer of cells in a zone of proliferative activity at the most medial portion of the lateral ventricles, and as the sling becomes smaller in older brains, it can be seen only near the ventricles. After 25.5 days, the sting cannot be seen in any brain.

It should be noted that there are substantial individual differences in CC shape among animals having the same age and CC area. As pointed out in previous reports, there are many adult BALB/c mice with a CC of normal cross-sectional area that is unusually short and thick compared to other strains, The CC of these adult mice resembles closely the shape of CC at 25.5 days in Fig. 2. What is virtually never seen at 25.5 days is a long and thin CC that is commonly seen in adults.

Phase 2

Among the 35 BALB mice in the present study and the 54 BALB mice from a previous study observed at 18.5 days, there were several which clearly had an abnormally small CC, but a criterion for abnormality was not immediately obvious. Inspection of the frequency distribution of CC area for the 89 BALB mice at 18.5 days as well as scores for animals at adjacent ages suggested that a CC with cross-sectional area less than 0.08 mm^2 was abnormally small at 18.5 days. However, there was evidence that a mouse might occur below this criterion for more than one reason. Criteria were therefore derived from data for 55 $B6D2F_2$ hybrid fetuses having nearly the same mean and range for whole brain and CC sizes. Linear regression of hybrid CC area on brain weight yielded the line of best fit shown in Fig. 4 with a goodness of fit of 0.766, and a one-sided 97.5% confidence interval was constructed with respect to this line. A mouse located below this line was considered to have CC area abnormally small for its brain size, whereas an animal with CC area less than 0.08 mm^2 but above the 95% confidence limit had CC size congruent with its brain size. In the latter case CC was small because growth of the whole brain was retarded. Thus, small CC occurred at 18.5 days for two reasons, one specific to CC growth and the other causing retarded growth of CC and whole brain alike. There were a few mice with CC area greater than 0.08 mrn^2 whose CC was nonetheless small for their brain sizes. This analysis of CC area was also done with respect to CA area and body weight, and results were essentially the same because CA area and body weight were highly correlated with brain weight among mice at 18.5 days.

The frequency of abnormally small CC among 18.5 day BALB mice was quite high, amounting to 20 instances of CC area less than 0.08 inm^2 and 22 instances of CC area below the 95% confidence limit among the 89 fetuses. Total absence of CC occurred 9 times, a frequency of about 10%. There were reasons to believe that a substantial portion of fetuses judged to have abnormally small CC at 18.5 days would recover and be judged normal had they been allowed to grow to maturity. First of all, among the 121 mice observed following birth in the first phase of the study, only one had total absence of CC, which is significantly different from the frequency of total absense at 18.5 days ($z = 2.82$, $P = 0.005$, two-tailed). Data were also available on 182 adult mice which had been produced by littermates and cousins of the mothers used in the present study in the same generation of an ongoing breeding program. Among these adult animals which were perfused at an age of at least 115 days after birth, only 6 had total absence of CC and another 19 had abnormally small CC size according to criteria derived previously for adult mice[22]. Data for these adult relatives of 18.5 day fetuses were used to derive expected frequencies of absent and small CC, weighting the observed frequency of absent or abnormal CC among adults by the number of fetuses obtained from the same family at 18.5 days. This procedure yielded an expected frequency of 3.5% total absence of CC, which was significantly less than the observed frequency at 18.5 days ($z = 3.12$, $P = 0.002$, two-tailed). Likewise, there were significantly more 18.5

day fetuses with abnormally small CC than the expected frequency of 14.2% (z = 2.38, $P = 0.017$, two-tailed). These results strongly imply that many mice with absent or small CC one day prior to birth eventually catch up with their litter-mates after birth.

Phase 3

There were 151 animals from 19 litters alive and marked when first observed on the day of birth. Among the 8 animals with birth weights below 1.15 g, which presumably were developmentally retarded one day prior to birth, half died prior to testing**,** whereas among the 143 mice weighing more than 1.15 g at birth, only 3 died. When histology was done on the brains of mice surviving 12 clays or more after birth, only 3 of 144 showed total absence of CC and another 10 showed abnormally small CC. These frequencies of absent and small CC were far below those noted at one day before birth, For those mice tested at either 12, 22 or 100 clays after birth, there were no significant differences between those having normal and abnormally small CC with regard to CA area, brain weight, body weight or birth weight (all $P > 0.05$, two-tailed t-tests). For mice tested at 12 days, neither mean reflex scores nor degree of myelination differed significantly between those with normal and abnormal CC. Associations among variables were evaluated separately for males and females with the Spearman correlation between ranks be cause some measures, especially CC area, were markedly skewed. At 12 days the animals which had been larger at birth were significantly more mature in brain and behavioral development *(*$P < 0.05$, one-tailed), but the brain weight—birth weight correlation was not significant in adult mice *(*$P > 0.05$). This indicates that most animals retarded at birth eventually catch up in brain development if they survive the neonatal period. The lack of significant association between CC size and birth weight even at 12 days after birth was probably a consequence of the early completion of the CC growth spurt. Because CC size reaches the adult range and growth decelerates about 7 days after birth, a mouse retarded by 1 or 2 clays would have ample time to catch up with normal littermates in CC size by 12 clays, although other measures of brain maturity might still be behind schedule. In any event, the occurrence of abnormally small CC in mice at 12 clays or more after birth does not have a significant relationship with retardation of development of the whole animal.

DISCUSSION

In BALB mice the anterior commissure crosses midplane well before the corpus callosum does so, as reported previously[21], but both of these forebrain fibre tracts increase rapidly in cross-sectional area and reach the adult range of size by 1 week after birth. The hippocampal commissure also shows rapid perinatal growth, although it was not measured in the present study because its rostra] edge is very difficult to visualize with these histological methods. The growth of these fibre tracts is precocial compared to the slower growth of the whole brain and is in the main completed prior to the onset of myelination of these tracts. This rapid phase of CC growth seems to occur just before the pioneering axons enter the intermediate layers of the cerebral cortex, although the studies of early interhemispheric connections need to be replicated with mice before strong conclusions are made. The corpus callosum continues to increase gradually in size for many months in mice[22], an increase which certainly reflects the growing proportion of axons with myelin sheaths and the higher numbers of lamellae in the sheaths[14].

The precise relation between cross-sectional area of CC and numbers of axons in this structure remains to be elucidated using electron microscopy and laborious axon counts. This effort will be complicated by the extreme shrinkage of the tissue which usually occurs during the dehydration process required for plastic embedding. The question of axon numbers in CC is of some interest because of findings that many interhemispheric connections evident in the early postnatal period cannot be detected in adult rats using tract-tracing methods[5,11] and that many axons are lost from the CC postnatally in the cat[9]. It is possible that the number of axons in CC actually decreases during the late neonatal period while CC cross-sectional area continues to increase because of myelination.

To what extent the different components of CC grow at different rates in the late postnatal and early prenatal periods remains to be determined using methods which can unambiguously define borders of the splenium and germ of CC. This will certainly require the use of tract-tracing methods to identify the splenium, for example,

by the region of cerebral cortex from which CC axons emanate. Sometimes strong statements about size of the splenium are made using unstained material[1], but such claims would seem to be unwise in view of the impossibility of drawing a line at the rostral limit of the splenium even in adult tissue clearly differentiated by a good myelin stain.

The methods used in the present study do not allow detection of the earliest pioneering axons of CC, but they do provide evidence concerning the glial 'sling' whose role in formation of the CC has been disputed[27,18]. This structure was apparent in every normal BALB brain examined in this study from the ages of 17,0-20,5 days. It was also evident in an earlier study of prenatal mouse brain development[21] clone with paraffin embedding and can in fact be seen clearly in Fig. 6B of that report. A recent report[7] provided evidence of the sling in fetal rat brain as well. It is possible that in rodents a few CC axons may traverse the hemispheres just above the hippocampal commissure in the absence of the glial sling, but in mice a normal sling appears to be necessary for formation of a normal CC rostral to the hippocampal commissure.

The present results indicate that absence or deficiency of corpus callosum in BALB mice is mainly attributable to mechanisms operative before birth. This is obvious in the case of total absence of CC because the structure normally appears two days prior to birth in BALB mice. However, the problem is not simply a malfunction which does or does not occur prior to 17 days gestation age. In some mice which lack a CC at 18.5 days gestation age a substantial CC must form belatedly. Silver et al. [13] reported that fusion of the cerebral hemispheres in the septal region is delayed in BALB mice which lack a CC prenatal)}. Thus, a good working hypothesis about the origin of CC defects in BALB mice can now be formulated. Perhaps the process of fusion of the hemispheres and hence the formation of the glial sling is retarded in many BALB mice relative to development of other forebrain structures, especially the neurons of the cerebral cortex, and the degree of retardation varies between individuals. The axons destined to form the CC then arrive in the vicinity of midplane on schedule but cannot cross until fusion of the hemispheres occurs. If the delay in fusion is small, then the axons are able to cross before any gross abnormality in structure occurs, although these brains with a small delay of fusion may be the ones which have a CC with the unusual shape, being short but thick, that is often seen in adult BALB brains. If the delay is substantial, many axons may eventually be able to cross midplane when fusion does occur, but others may have been diverted longitudinally in the meantime, leading to an adult animal with a small CC. If the delay in fusion is too long, most of the putative CC axons may have begun to course longitudinally to form the Probst bundle which is characteristic of total absence of CC in the adult[8,10]. That is, the degree of deficiency of CC in the adult may reflect the degree of delay in fusion of the hemispheres in the fetal brain, and the problem of agenesis of CC, which most certainly involves several distinct physiological processes, may be a defect of the spatio-temporal coordination of development of different parts of the brain[12], similar in sonic ways to another hereditary defect of mouse morphology, the cleft palate[16].

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